

ABSTRACT

[00129] The present invention provides a method of cDNA sequencing by circulating subtraction, which comprises the steps of: (1) constructing the cDNA original libraries with high quality using the organism tissues as materials; (2) homogenizing the cDNA original libraries of step (1) according to the graded  $C_{0t}$  value, thereby obtaining the homogenized cDNA libraries corresponding to the graded  $C_{0t}$  value; (3) selecting and sequencing the clones from each of the homogenized cDNA libraries of the preceding step; (4) hybridizing and subtracting the homogenized cDNA libraries of the preceding step by using the sequenced cDNA clones; (5) repeating steps (3) and (4) 1-5,000 times. Further, a step of preliminary subtractive hybridization may be included in the method. By using said method, one can sequence cDNA of certain tissues or species efficiently and comprehensively.